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Goat and Sheep Fecal Matter Mediated Biological Synthesis of Zinc Oxide Nanoparticles (ZnONPs) and their Biological Activities

Shivayogeeswar E Neelagund*, Mahesh Midatharahalli Chikkanna, Kotresh K Rajashekarappa

Department of Studies and Research in Biochemistry, Jnana Sahyadri, Kuvempu University, Shankaraghatta, Shimoga, Karnataka-577 451, India

ABSTRACT

Since various attempts have been made in the field of nanoparticle synthesis by different methods, biological synthesis gain main attention in this regard due to its low cost, less usage of chemicals, one and helpful to gain more yield. In this paper we have used sheep and goat fecal matter as reducing agent to reduce zinc sulfate to Zinc Oxide Nanoparticles (ZnONPs). Synthesized ZnONPs were subjected to characterization using UV-Visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray Diffraction (XRD) and Scanning Electron Microscope (SEM) analysis. XRD and SEM analysis were used to determine the average crystallite size, structure and shape of the ZnONPs. FTIR analysis indirectly provides composition and purity of the sample and formation of ZnONPs. The antimicrobial activity was performed against pathogenic organisms like gram negative Salmonella typhimurium, and gram positive Bacillus subtilis. These tests have shown excellent results against both organisms.

Keywords: ZnONPs, Goat and sheep fecal matter, Biological synthesis, ZnONPs synthesized using Sheep Fecal Matter (SFM), ZnONPs synthesized using Goat Fecal Matter (GFM)

INTRODUCTION

The main aim of researcher is to achieve some great heights and to innovate new ideas in biological field. Nanotechnology, a recently developing and getting attain in the field of research, because of its nature and size which has been proved in many more experiments. The science and engineering technology of nano-systems is one of the most exigent and fastest growing sectors of nanotechnology [1]. Nature has devised various processes for the synthesis of nano and micro- length scaled inorganic materials which have contributed to the development of relatively new and largely unexplored area of research based on the biosynthesis of nanomaterials. Synthesis using bio-organisms is congruent with the green chemistry principles. "Green Synthesis" of nanoparticles makes use of environmental friendly, non-toxic and safe reagents. Zinc Oxide Nanoparticles (ZnONPs) belongs to the class of metal oxides, which is characterized by photo catalytic and photo-oxidizing capacity against chemical and biological species [2]. There are many more nanoparticles in this field such as silver, zinc, copper, gold etc. ZnONPs gains much more attention in various fields. Green synthesis techniques make use of moderately pollutant free chemicals to synthesis nanomaterials and embrace the use of benign solvents such as water, natural extracts. Green chemistry seeks to reduce pollution at source [3,4]. Nanoparticles due to their smaller size and large surface to volume ratio exhibit remarkable novel properties and methodical applications in the field of biotechnology, sensors, medical, catalysis, optical devices, DNA labeling, drug delivery [5].

Most of the synthetic physicochemical methods reported till date are heavily on the use of organic solvents and toxic reducing agents like thiophenol, mercapto acetate, sodium borohydride etc. Most of these chemicals are highly reactive and pose potential environmental and biological risks. With the increasing interest in minimization or elimination of such kinds of hazardous chemicals, the development of biological, biomimetic and biochemical approaches is desirable. Therefore, biological approach has advantages over physicochemical methods because of its clean, non-toxic chemicals, environmentally benign solvents, and user-friendly nature [6].

Sheep and goat fecal matter have greater application in agricultural field as manure, rather than that they are considered as waste material, so that we have chosen them as reducing agents in nanoparticle synthesis. In this present work we have synthesized zinc oxide nanoparticles by biological synthesis method.

EXPERIMENTAL SECTION

Materials and methods

All materials and chemicals were used as received. Zinc sulphate (M.W 287.54) was purchased from Jain chemicals, Shimoga, Karnataka, India. Fecal matters were collected from local area of Madhugiri, which were collected, washed with distilled water, shade dried in room temperature and kept in sealed cover for further use.

Extract preparation

20 g of dried fecal matters were taken in different container, crushed in pestle mortar for powder and mix with 100 ml of distilled water. Mix thoroughly and kept microwave heat for 3 times for 3 min under a power of 90W. After cooling, filtered with Whatman filter paper No. 1, the filtrates were used as reducing agent for nanoparticle synthesis.

Synthesis of zinc oxide nanoparticles

10 ml of goat and sheep fecal matter extracts were added to 2 different conical flasks containing 100 ml of 0.1 M Zinc Sulphate ($ZnSO_4$) solution. pH of the solution was maintained to 8 to attain smaller size particles. The solutions were kept on magnetic stirrer for one hour, the solution turns into milky white indicates the particles formation.

Synthesized particles were subjected to further washing with distilled water for several times, with solvents for removal of impurities along with nanoparticles and further particles kept in oven for heat drying at 100°C to remove other impurities, which converts zinc nanoparticles to zinc oxide nanoparticles and also slightly increase in the particles size.

Particle characterization

Synthesized particles were characterized for further confirmation of particle synthesis. The absorption spectrum was measured by using UV-Visible spectrophotometer (HR 4000 UV-Visible spectrophotometer, UV-Vis-NIR light source, DT-MINI-2-GS, Jaz detector). The average particle size and phase detection of particles were evaluated by X-ray Diffraction (XRD) pattern using X'pert Pro diffractometer (Phillips, Cu-Kα radiation, λCu=1.5148 Å) working at 30 mA and 40 kV recorded in the 2θ range between 10° and 90° (scan rate 1° min^{-1}). Morphological features were studied by using Philips XL30 scanning electron microscope (SEM).

Antibacterial activity

Bacterial strains of gram positive *Bacillus subtilis* (ATCC 19659) and gram negative *Salmonella typhimurium* (ATCC 23564) were used to determine antibacterial activity by agar well diffusion method. Nutrient agar medium was used as culture, 100 µl of 24 h old mature cultures were swabbed using L-shaped rod on medium. Wells were made using sterile cork borer (6 mm). ZnONPs were dispersed with Dimethyl Sulfoxide (DMSO $(CH_3)_2SO$, mm 78.13 g/mol.) for well dispersion of nanoparticles, which was used as control. Ampicillin ($C_{16}H_{19}N_3O_4S$, M.W. 349.41 g/mol.) was used as standard. Zone of Inhibition (ZOI) was measured in mm. Four wells were made in each Petri plate and were filled with 50 µl of DMSO, ampicillin (100 µg/ml), ZnONPs (GFM) (100 µg/ml) and ZnONPs (SFM) (100 µg/ml) respectively, GFM and SFM extracts were tested in different plates against both organisms.

RESULTS AND DISCUSSION

UV-Visible spectrophotometer analysis of ZnONPs (GFM) and ZnONPs (SFM)

Synthesized nanoparticles were subjected to UV-Visible spectrophotometer analysis which gave us the idea of nanoparticle formation in the first step itself. The solid white colored samples of ZnONPs synthesized using both sheep and goat fecal matters were subjected to scan UV-Spectrophotometer in the range of 200-1000 nm. Various peaks were observed under UV region, for nanoparticles synthesized using goat fecal matter and sheep fecal matter as reducing agents which were recorded in Table 1 indicates the zinc oxide nanoparticles formation. Peaks at 352.67 nm and 356.60 nm indicates the zinc oxide nanoparticles formation using goat fecal matter and peaks from 352.60 nm and 353.67 nm indicates the zinc oxide nanoparticles formation [7] using sheep fecal matter as reducing agent. Figure 1a and 1b reveals the UV-Visible characterization peaks of zinc oxide nanoparticles.

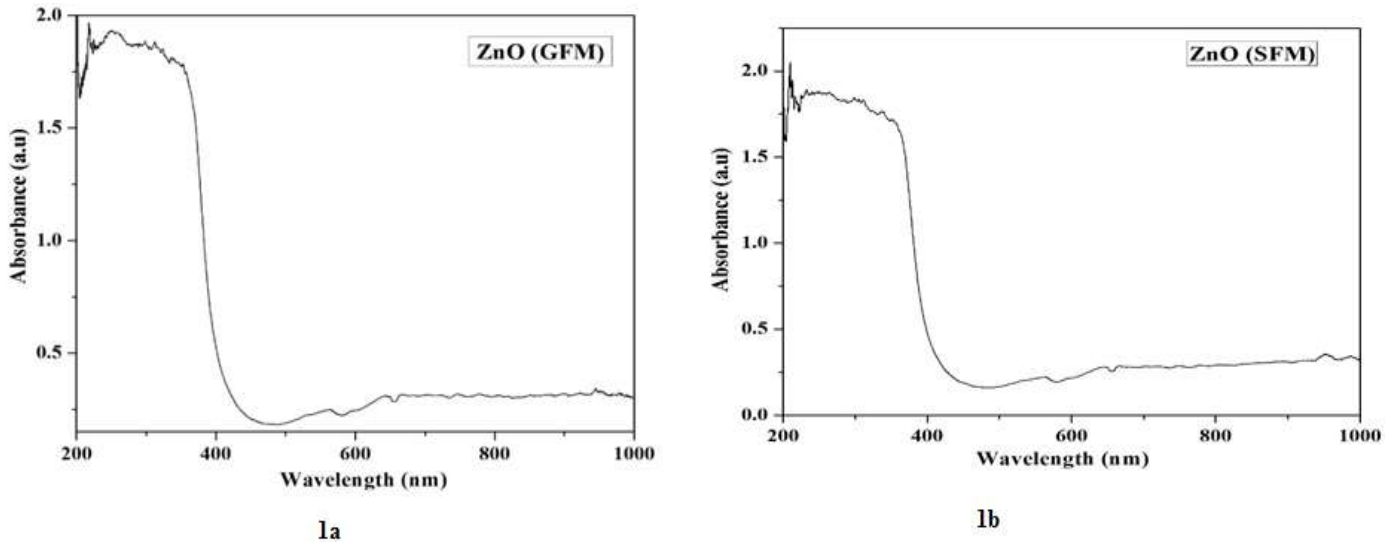


Figure 1: (1a) UV-Visible absorption spectrum of ZnONPs (GFM), (1b) UV-Visible absorption spectrum of ZnONPs (SFM)

Table 1: UV-Visible absorption values of zinc oxide nanoparticles synthesized using goat and sheep fecal matter

UV-Visible absorption for ZnONPs (GFM)	UV-Visible absorption for ZnONPs (SFM)
217.45 nm	213.87 nm
298.57 nm	224.75 nm
304.64 nm	232.58 nm
312.66 nm	298.28 nm
316.07 nm	304.82 nm
324.23 nm	311.33 nm
335.96 nm	316.98 nm
339.88 nm	323.97 nm
346.08 nm	335.25 nm
352.60 nm	337.45 nm
353.57 nm	339.62 nm
--	351.38 nm
--	352.67 nm
--	356.60 nm

XRD pattern for ZnONPs synthesized using goat and sheep fecal matter as reducing agents

Synthesized particles were undergone for X-Ray diffraction studies, to obtain the crystallinity and average particle size of synthesized nanoparticles. Figure 2a and 2b reveals the XRD pattern of zinc oxide nanoparticles. Number of Bragg reflections for ZnONPs using goat fecal matter appears at 2θ values and corresponding Miller indices values were recorded in Table 2. Peaks for ZnONPs synthesized using sheep goat fecal matter appears at 2θ values corresponding Miller indices values were recorded in Table 3. The planes shows a good agreement with JCPDS file (JCPDS: 80-0075 card ICSD#: 067849), which indexed the hexagonal wurtzite structure which corresponds to pure zinc oxide nanoparticles [8,9]. By using Debey-Scherrer equation [10], average particle size of synthesized nanoparticles were calculated to be as 28.5 nm for ZnONPs (GFM) and 24.4 nm for ZnONPs (SFM). The broad peak indicates the decreasing in crystallinity, which inwards suggests the formation of smaller particles size. Figure 2a and 2b reveals the XRD pattern of ZnONPs (GFM) and ZnONPs (SFM).

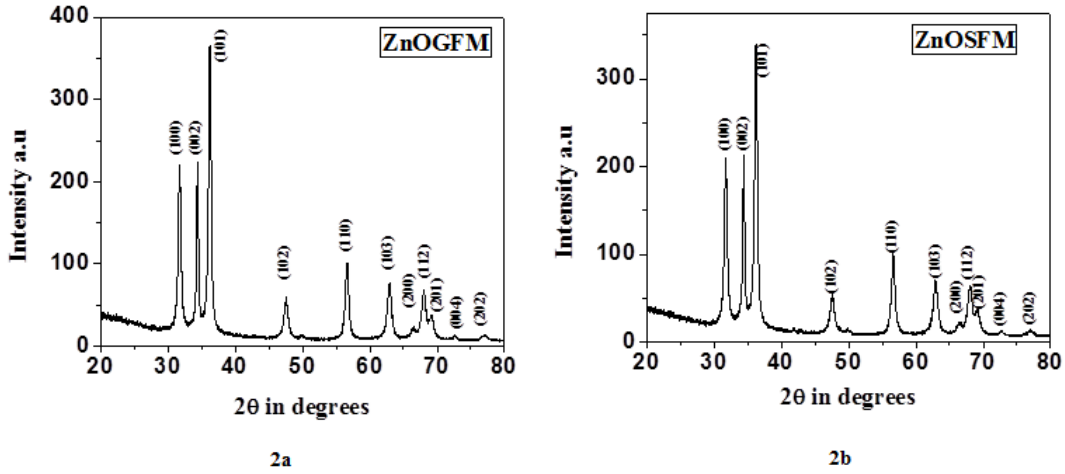


Figure 2: (2a) XRD pattern of ZnONPs (GFM); (2b) XRD pattern of ZnONPs (SFM)

Table 2: XRD values of zinc oxide nanoparticles synthesized using goat fecal matter

2θ values	Hkl values
31.77°	(100)
34.44°	(002)
36.27°	(101)
47.62°	(102)
56.73°	(110)
62.96°	(103)
68.06°	(112)
69.13°	(201)
77.04°	(202)

Table 3: XRD values of zinc oxide nanoparticles synthesized using fecal matter

2θ values	Hkl values
31.79°	(100)
34.47°	(002)
36.26°	(101)
47.67°	(102)
56.66°	(110)
62.94°	(103)
68.01°	(112)
69.19°	(201)
77.04°	(202)

FTIR analysis of synthesized zinc oxide nanoparticles

The Fourier Transform Infrared Spectra (FTIR) were analysed using FTIR JASCO FTIR-5300 model, in the range of 0-4000 cm^{-1} was recorded using KBr pellet method to recognize the organic, inorganic, biomolecule residues along with nanoparticle formation, which may come along via reducing agent on to the surface of ZnONPs. Absorption bands for ZnONPs (GFM) and ZnONPs (SFM) were recorded in Table 4. The intense broad bands at 3445.55 cm^{-1} and 3417.63 cm^{-1} were assigned to O-H stretching of flavonoids, polyphenols and C-O groups on the surface of ZnO crystal nano structure which may present in fecal matter extract that indicates the bending frequencies of H_2O reveals the water content on the surface of nanoparticles [11]. The intense peaks at 2334.10 cm^{-1}, 2361.74 cm^{-1} and 2340.96 cm^{-1} indicates the formation of CO_2 molecules which may absorb during synthesis [12]. The peak at 1630.06 cm^{-1} is corresponding to C=C stretch in the aromatic ring. Bands at 1099.15 cm^{-1} and 937.71 cm^{-1} indicates the stretching of C-O in the amino acid [13]. Bulk peaks at 672.03 cm^{-1}, 537.92 cm^{-1} and 498.51 cm^{-1} indicates the significance presence of R-CH group which may be the possible inclusion with fecal matter residues [15], peaks at 464.51 cm^{-1} for ZnO-GFM (Figure 3a) and peaks at 433.79 cm^{-1} and 461.27 cm^{-1} for ZnO-SFM (Figure 3b) indicates the stretching vibrations of zinc and oxygen bonds, which shows the formation of ZnO nanoparticles [15-17].

Regardless of repeated washing the surveillance proves the subsistence of aldehydes, amines, terpenoids and phenolic compounds were bounded to the surface of ZnONPs enhances the stabilization by covering the metallic nanoparticles [18].

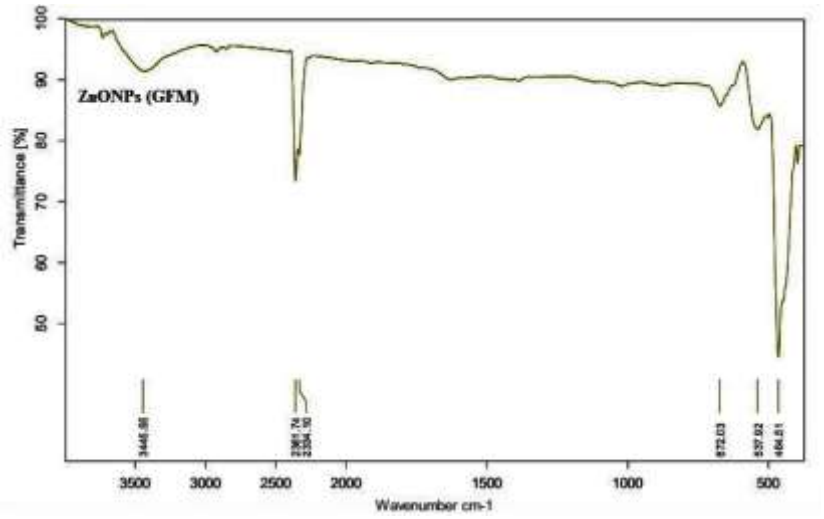


Figure 3a: FTIR spectra of ZnONPs synthesized using goat fecal matter as reducing agent

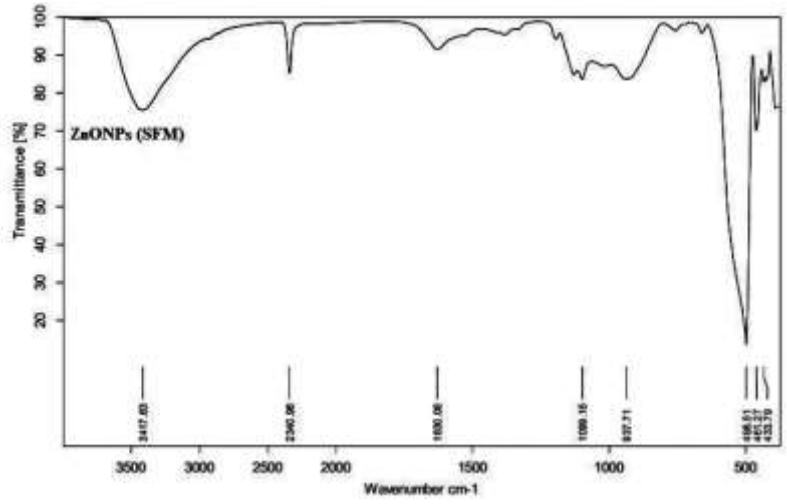


Figure 3b: FTIR spectra of ZnONPs synthesized using sheep fecal matter as reducing agent

Table 4: FTIR absorption values of ZnONPs (GFM) and ZnONPs (SFM)

Absorption values of ZnONPs (GFM)	Absorption values of ZnONPs (SFM)
3445.55 cm^{-1}	3417.63 cm^{-1}
2361.74 cm^{-1}	2340.96 cm^{-1}
2334.10 cm^{-1}	1630.06 cm^{-1}
672.03 cm^{-1}	1099.15 cm^{-1}
537.92 cm^{-1}	937.71 cm^{-1}
464.51 cm^{-1}	498.51 cm^{-1}
--	461.27 cm^{-1}
--	433.79 cm^{-1}

Scanning Electron Microscope (SEM) analysis

SEM analysis is used to analyze the structural and morphological confirmation of synthesized nanoparticles Figure 4a and 4b and Figure 5a and 5b reveals the SEM images of ZnONPs (GFM) and ZnONPs (SFM). Particles clearly execute the spherical structural formation. In following images we can clearly observe the obtained particles possess nearly spongy like and flower like structural nanoparticles. The particles were initially seemed to be as flakes at initial SEM studies (Figure 4a and 4b). Following Figure 4a and 4b there were some small particles which observed to be bit agglomerated, which possess some higher particle sizes as nearly 91-180 nm range. Whereas, in Figure 5a and 5b we can clearly observe oval like structured particles, seemed to have flower like structure, has lower particle size when compared to Figure 4a and 4b as nearly 40-120 nm range. A homogenous distribution of particles can give us better knowledge on morphological study and approximate particles size.

From the literature survey [19] it was found that usage of natural particles as reducing agents sometimes leads to particles agglomeration and somewhat particles will seems to have bit bigger particles size, correspondence to ZnO nanoparticles by biological synthesis [20].

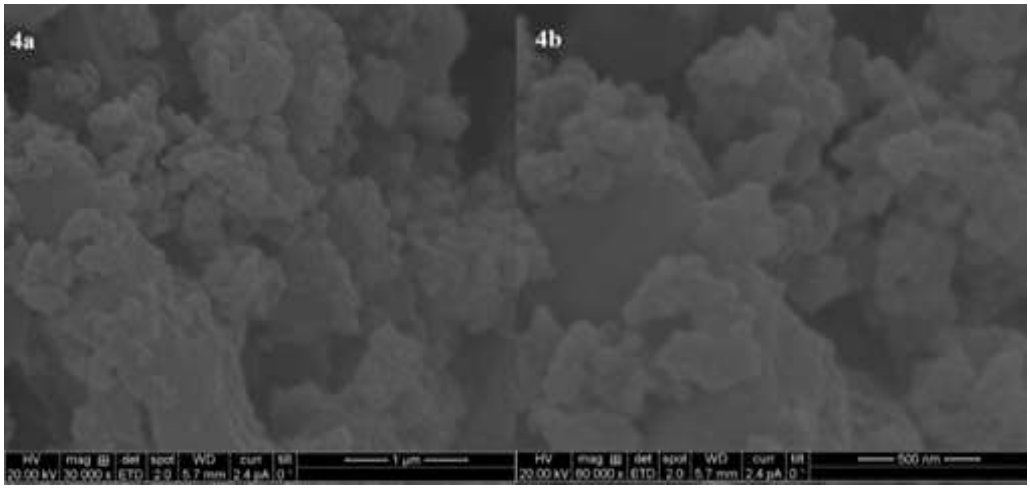


Figure 4: 4a and 4b shows the SEM images of zinc oxide nanoparticles synthesized using goat fecal matter

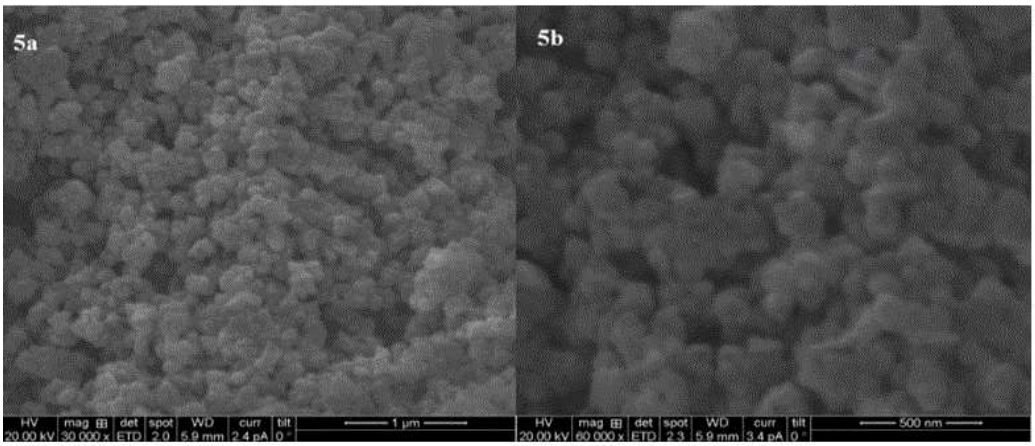


Figure 5: 5a and 5b shows the SEM images of zinc oxide nanoparticles synthesized using sheep fecal matter

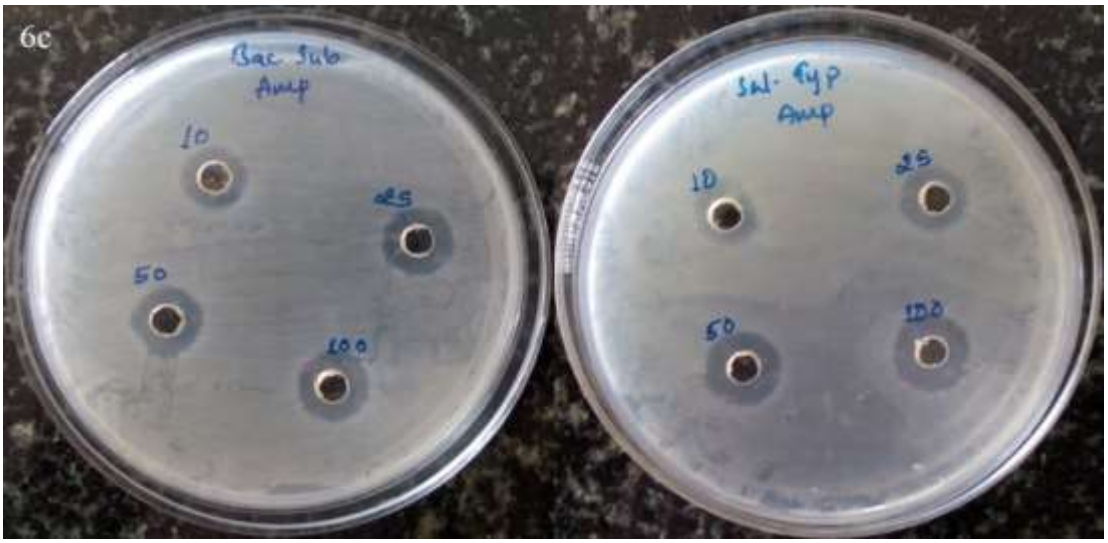
ZOI and MIC of ZnONPs

Synthesized nanoparticles were subjected to antimicrobial activity. Particles were tested against both Gram-positive *B. subtilis* and Gram-negative *S. typhimurium* using agar well plate method [21]. Secondary cultures were made using primary culture and 24 h old secondary cultures were used for activity. No ZOI was observed for DMSO for both organisms. Radial diameter of ZOI for DMSO, ZnONPs (GFM), ZnONPs (SFM) and ampicillin against *S. typhi*, *B. subtilis* were recorded in Table 5. It shows significantly an average ZOI for ZnONPs against both organisms when compared with standard ampicillin. Based on these results, it could be conclude that these synthesized ZnONPs has shown significant antibacterial activity on both of the gram classes of bacteria. This antibacterial activity may be attributed due to the presence of amines and carboxyl groups on their cell surface and occurrence of greater affinity of zinc oxide ions toward these groups [22]. ZnONPs has shown efficient antibacterial property due to their extremely large surface area, which could provide better contact with microorganisms. Zinc ions released subsequently may be bind with DNA molecules and lead to disorder of the helical structure by cross-linking within and between the nucleic acid strands. Zinc ions inside bacterial cells were also involved in disruption of biochemical processes [23,24]. The antibacterial activity of ZnONPs towards Gram-negative bacteria was observed higher when compared to Gram-positive bacteria. The difference in activity against these two types of bacteria could be attributed to the structural and compositional differences in the cell membrane [25]. Gram-positive bacteria have thicker peptidoglycan cell membranes compared to the Gram-negative bacteria and it is harder for ZnONPs to penetrate it, resulting in a low antibacterial response [26].

Minimum Inhibitory Concentration [MIC] was also calculated against both organisms using different concentration of samples. MIC values of ZnONPs (GFM), ZnONPs (SFM) and ampicillin against *S. typhi* were recorded in Table 6. MIC values of ZnONPs (GFM), ZnONPs (SFM) and ampicillin against *B. subtilis* were recorded in Table 7. By MIC we can notice gradual increase in zone of inhibition by increasing in

concentration of nanoparticles which indicates the effect of nanoparticles on organisms. We have observed that very low concentration of ZnO didn't shown any activity against bacterial strains (Figure 6a-6e), which may be due to presence of lesser zinc oxide nanoparticles present sometimes act as nutrient to organisms. Antimicrobial activity is always higher above 5 µg/ml concentration [27].

Considering previous results [18,28-30], we can report our obtained results have shown more activity. From MIC and ZOI we can clearly noticed that ZnO synthesized using green synthesis always possess smaller particle size have great antibacterial effects due to larger surface area to volume ratio and surface reactivity. Numbers of studies have done on the considerable impact of particle size on the antibacterial activity and the researchers found that controlling ZnO-NPs size was crucial to achieve best bactericidal response, and ZnO-NPs with smaller size (Higher specific surface areas) showed highest antibacterial activity [31-33]. ZnONPs prepared from green synthesis method is more effective than other mode of preparation, which may be due to oxygen species released on the surface of ZnO, which causes fatal damage to microorganisms [34]. The dissolution of ZnONPs into Zn^{2+} was reported as size dependent, and few studies suggested this dissolution of Zn^{2+} responsible for toxicity of ZnONPs. They react with hydrogen ions to produce molecules of H_2O_2. The generated H_2O_2 can penetrate the cell membrane and act lethal to microorganisms [35]. The generation of H_2O_2 depends strongly on the surface area of zinc oxide nanoparticles, which results in more oxygen species on the surface and the higher antibacterial activity of the smaller nanoparticles [36]. The effect of size and concentration was successfully analyzed by Padmavathy and Vijayaraghavan [37] who described the generation of H_2O_2, which depends mainly on the surface area of ZnO. The larger the surface area and the higher concentration of oxygen species on the surface can obtain greater antibacterial activity by smaller particles, which was in contrast to that of Franklin et al., [38] who found no size-related effect. In general, a correspondence between nanoparticles size and bacteria appears to be required for the bioactivity of ZnONPs, as well the concentration.



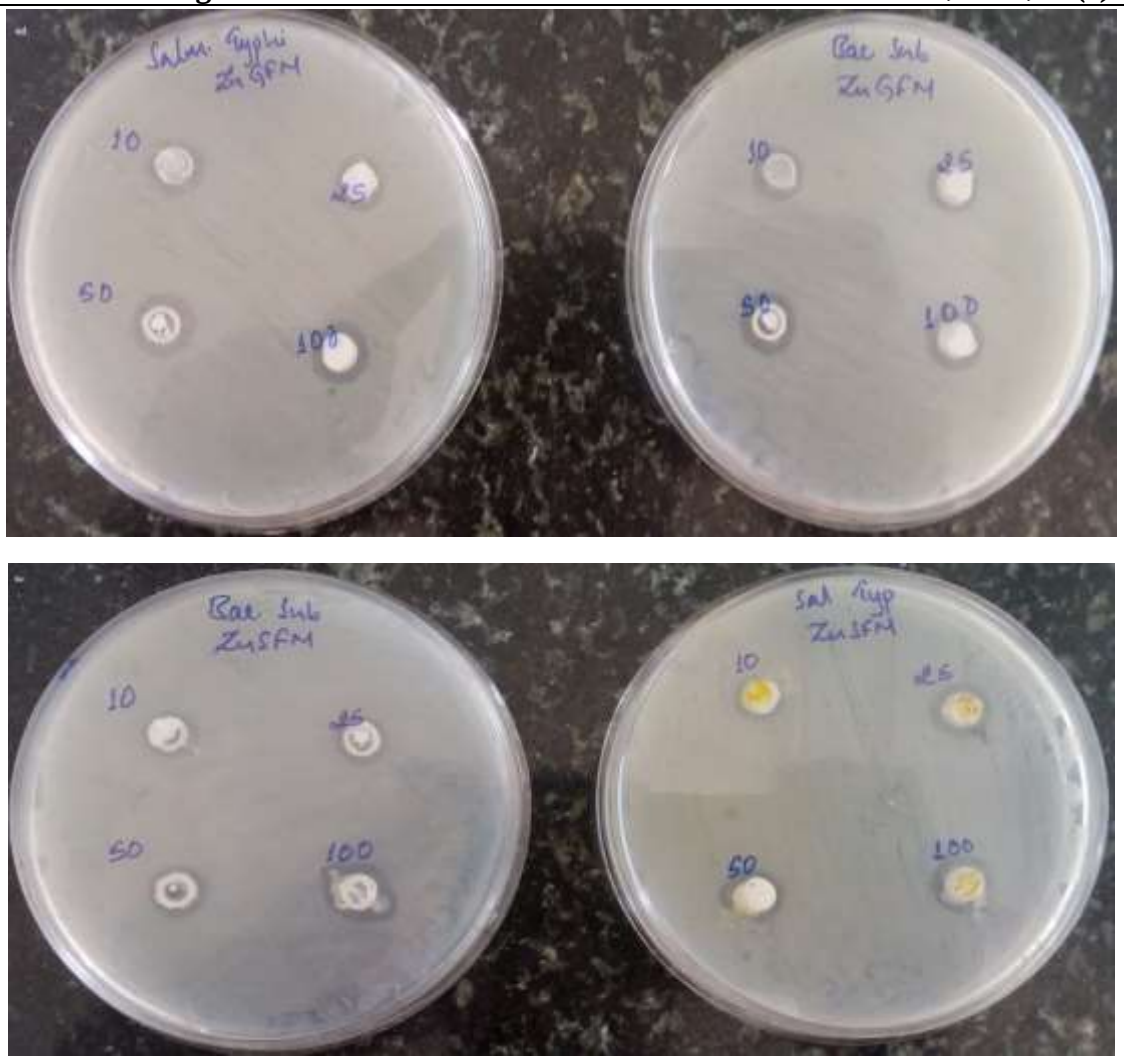


Figure 6: (a) Zone of inhibition of DMSO, ampicillin and ZnONP (GFM) and ZnONP (SFM) against *Salmonella typhimurium,* (b) Zone of inhibition of DMSO, ampicillin and ZnONP (GFM) and ZnONP (SFM) against *Bacillus subtilis,* (c) MIC of ampicillin, right plate shows against *S. typhimurium* and left plate shows activity against *B. subtilis*, (d) MIC of ZnONPs (GFM), right plate shows activity against *S. typhimurium* and left plate shows activity against *B. subtilis* € MIC of ZnONPs (SFM) (e) right plate shows activity against *B. subtilis* and left plate shows activity against *S. typhimurium*

Table 5: ZOI of zinc oxide nanoparticles synthesized using goat and sheep fecal matter as reducing agent against *S. typhimurium* and *B. subtilis*

Bacterial strain	ZnONPs (GFM) (mm)	ZnONPs (SFM) (mm)	Ampicillin (mm)
Salmonella typhimurium	3.23 ± 0.15	2.93 ± 0.15	4.43 ± 0.05
Bacillus subtilis	2.46 ± 0.06	2.1 ± 0.10	3.16 ± 0.05

Table 6: MIC values of ZnONPs (GFM), ZnONPs (SFM) and ampicillin against *S. typhimurium*

Conc.(µg)	ZnONPs (GFM) (mm)	ZnONPs (SFM) (mm)	Ampicillin (mm)
10	1.16 ± 0.15	0.96 ± 0.14	2.12 ± 0.10
25	1.56 ± 0.05	1.63 ± 0.07	2.43 ± 0.13
50	2.38 ± 0.15	2.18 ± 0.17	3.35 ± 0.13
100	3.2 ± 0.20	3.15 ± 0.17	4.34 ± 0.11

Table 7: MIC values of ZnONPs (GFM), ZnONPs (SFM) and ampicillin against *B. subtilis*

Conc.(µg)	ZnONPs (GFM) (mm)	ZnONPs (SFM) (mm)	Ampicillin (mm)
10	1.08 ± 0.24	1.15 ± 0.14	1.96 ± 0.15
25	2.01 ± 0.22	1.5 ± 0.07	2.2 ± 0.13
50	2.5 ± 0.10	2.18 ± 0.17	3.15 ± 0.08
100	2.98 ± 0.22	3.43 ± 0.21	3.8 ± 0.13

CONCLUSION

From this study we have showed green synthesis of zinc oxide nanoparticles using sheep and goat fecal matter as reducing agent, ends with giving a satisfactory results which were showed by their characterizations, UV at 352.67 nm for ZnONPs (GFM) and 362.50 nm for ZnONPs (SFM), average particles sizes were calculated as 28.5 nm for ZnONPs (GFM) and 24.4 nm for ZnONPs (SFM) FTIR results give us a better confirmation about the ZnONPs formation by forming an intense peaks at 537.92 nm and 464.51 nm for ZnO-GFM and peaks at 433.79 nm, 461.27 nm and 498.51 nm for ZnO-SFM. SEM gave us the structural images of particles as spongy like particles for ZnONPs (GFM) and flower

like structures for particles for ZnONPs (SFM). Furthermore, we have subjected to antibacterial activity against both gram positive and gram negative organisms, and obtained a remarkable activity against both.

No harmful chemicals or reagents were used in this experiment, gives a very good result as optimum sized nanoparticles and leads us to reducing in using of chemicals which in terms help us to look out for usage of natural waste products. In future we can refer this work as best example for upcoming projects, look out for low cost and more efficient particles, which may help in drug targeting and particular disease curative agents for both plants and animals.

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